

## UDDER-HALVE-WISE COMPARATIVE PREVALENCE OF CLINICAL AND SUB-CLINICAL MASTITIS IN LACTATING GOATS WITH THEIR BACTERIAL PATHOGENS AND ANTIBIOTIC SENSITIVITY PATTERNS IN BANGLADESH

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### ABSTRACT

Goats are the major parts of subsidiary economy of the rural people in Bangladesh. Dairy animals including lactating goats are prone to the intramammary infection (IMI) and associated with a lot of economic impact on the farmers but the reports on caprine mastitis are very limited in inland literature. This study was undertaken to determine the udder-halve-wise comparative prevalence of clinical and sub-clinical caprine mastitis with their associated bacterial pathogens and antibiotic sensitivity patterns during the period from January to May 2010. The teat and udder of a lactating population of 1025 Black Bengal goats maintained under rural (village) condition in two different districts (Joypurhat and Mymensingh) of Bangladesh were physically examined, of which 54 (5.27%) goats had clinical mastitis which constituted as experimental animals for this study. Of the 54 selected goats, 59 (54.63%) udder-halves were affected with clinical mastitis whereas the remaining 49 (45.37%) udder-halves of the selected goats were found physically normal. Out of 59 udder-halves, 49 (90.74%) were unilaterally and 5 (9.26%) were bilaterally affected with clinical mastitis. The prevalence of clinical mastitis was found significantly ( $p < 0.01$ ) higher in left udder-halves ( $H = 47$ ; 79.66%) in comparison to the right ( $H = 12$ ; 20.34%) udder-halves. Milk samples collected from all the 108 udder-halves were examined bacteriologically, of which 102 (94.44%) udder-halves had bacterial infection. No significant differences was observed on the status of bacterial pathogens between clinically ( $H = 55$ ; 93.22%) and sub-clinically ( $H = 47$ ; 95.92%) affected udder-halves, and between the single (CH = 45; 76.27% and SCH = 35; 71.43%) and mixed (CH = 10; 16.95% and SCH = 12; 24.49%) bacterial infections in both the clinical and sub-clinical mastitis udder-halves. The major bacterial pathogens isolated were *Staphylococcus* spp. which showed to be highest frequency (38.98%), followed by *Escherichia coli* (27.12%) and *Bacillus* spp. (10.17%) as a single clinical IMI whereas mixed IMI were also detected in both clinically and sub-clinically affected udder-halves. Antibiotic sensitivity results showed a relatively high level of resistance to ampicillin, amoxycillin and streptomycin, whereas gentamicin and ciprofloxacin were found to be the most effective drugs. These results indicate the high prevalence of sub-clinical mastitis in apparently normal quarters of lactating goats than those had clinical mastitis in other udder-halves, and therefore, there is a need for the prudent use of antibacterial in animal health and production through bacteriological and antibiogram studies in Bangladesh.

**Key words:** Lactating goats, Udder-halves-wise mastitis, Bacterial pathogens, Antibiotic sensitivity

### INTRODUCTION

Bangladesh possesses about 34 million goats and more than 90% of the goats of the country are Black Bengal breed and it provides 127,000 MT meat which accounts for 25% of total red meat in Bangladesh (FAO, 2007). The goat ranks second in position in terms of meat, milk and skin production, representing about 28, 23 and 28% respectively of the total livestock in Bangladesh. The comparative beneficial effects of goat milk with human and other livestock species have been described (Haenlein, 2004) but the goat is perhaps the most misunderstood and neglected species of livestock in Bangladesh. Although goat rearing is easy, less expensive, less laborious and highly profitable business, it is being seriously hampered due to various disease problems. Mastitis is one of the most important diseases of mammals including goats worldwide including Bangladesh (Bozhilov, 1970; Samad, 2008). Mastitis is the inflammation of mammary glands with physical, chemical and microbiological changes characterized by an increase in somatic cells, especially leukocytes in the milk and by the pathological changes in the mammary tissue. Generally, mastitis occurs in two forms which includes clinical (overt) and sub-clinical (hidden). In the clinical mastitis all the five cardinal signs of udder inflammation (redness, heat, swelling, pain and loss of milk production) are present, while the sub-clinical form is bereft of any obvious manifestation of inflammation. Mastitis is recognized as the most important and costly disease of dairy animals worldwide but also poses the risk for the transmission of milk borne zoonotic diseases (Samad, 2008). The distribution, causes, importance and antibiogram patterns of mastitis pathogens isolated from lactating goats have reported from

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different parts of world (Manser, 1986; Contreras *et al.*, 1995; Boscos *et al.*, 1996; Moshi *et al.*, 1998; Ameh *et al.*, 1993; Ameh and Tari, 2000; Ndegwa *et al.*, 2000; Egwu *et al.*, 1994; Bhujbal *et al.*, 1999; DaSilva *et al.*, 2004; Wakwoya *et al.*, 2006; Mbilu, 2007). However, the reports on caprine mastitis are very rare from Bangladesh where Rahman (1981) reported only the clinical occurrence and Rahman and Samad (1984) isolated *Staphylococcus aureus* from three clinical cases of caprine mastitis. Moreover, the greatest problem in the treatment and control of mastitis is the emergence of drug resistance due to indiscriminate use of antibacterial reported elsewhere (Jha *et al.*, 1994) which is remained unexplored in caprine mastitis in Bangladesh. This paper describes the udder-halves-wise comparative prevalence of clinical and sub-clinical mastitis in lactating Black Bengal goats with their associated bacterial pathogens and antibiotic sensitivity patterns.

## MATERIALS AND METHODS

The udder-halves-wise comparative prevalence of clinical and sub-clinical mastitis with their associated bacterial pathogens and their *in vitro* sensitivity patterns to different antibiotics were carried out in 54 Black Bengal goats in the districts of Joypurhat (n = 36) and Mymensingh (n = 18) during the period from January to May 2010. Clinical mastitis was diagnosed in 54 goats from a randomly selected population of 1025 lactating rural goats on the basis of owner history, inspection and palpation of the udder and visual examination of the milk samples. Hard swollen with painful udder and visible abnormalities of milk (milk with blood, clots, flakes, watery etc.) were diagnosed as clinical mastitis.

### Collection of milk samples

Udders and teats of the randomly selected 54 lactating goats (108 udder-halves) were cleaned and dried before sample collection. Each of the 59 mastitis affected teats and 49 apparently healthy teats of 54 lactating goats was disinfected with 70% ethanol before sampling. The first few streams of milk were discarded and about 10 ml milk samples were collected in sterile universal bottles, labeled as L or R (L for left halves and R for rights halves). The milk samples were transported in an icebox with ice to the laboratory for bacteriological studies.

### Isolation and identification of bacteria

The media and reagents were either obtained from Hi-Media, Mumbai, India or prepared in the laboratory as per the standard procedures (Cowan and Steel, 1974; Cruickshank *et al.*, 1975). Bacteriological examination of 108 milk samples collected from 108 quarters of 54 lactating goats, of which 59 quarters were affected with clinical mastitis was carried out by following standard procedures (Cruickshank *et al.*, 1975; Quinn *et al.*, 1999).

Milk samples were incubated aerobically at 37 °C for 24 hours. Then about 10 µl milk from each quarter was inoculated onto blood agar, MacConkey agar and nutrient agar plates and incubated aerobically at 37 °C for 24 to 48 hours. Each plate was then examined for growth, morphology and hemolytic characteristics. Identification of bacteria on primary culture was made on the basis of colony morphology, hemolytic characteristics, Gram-stain reaction and catalase tests. Further each culture was subjected to various biochemical tests (Kreig and Holt, 1984). Finally, the bacteria isolated were identified on the basis of their cultural, morphological and biochemical characteristics as per method of Cruickshank *et al.* (1975).

### Antibiotic susceptibility test

Antibiotic susceptibility test was conducted on randomly selected three different bacterial pathogens (*Staphylococcus* spp., *Escherichia coli* and *Bacillus* spp.) isolated during the study period to 10 antibiotics using the disc diffusion method (Ellner, 1978; Quinn *et al.*, 1999). Minimal inhibition concentration values of the bacterial organisms were analyzed for antibacterial discs (Oxoid Ltd., UK) with their corresponding concentrations include: Gentamicin (GN, 30 µg), Ciprofloxacin (CIP, 5 µg), Erythromycin (ERY, 15 µg), Chloramphenicol (C, 30 µg), Ampicillin (AMP, 10 µg), Amoxicillin (AMOX, 10 µg), Enrofloxacin (ENR, 5 µg), Oxytetracycline (OXT, 30 µg), Doxycycline (DOX, 30 µg) and Streptomycin (SM, 10 µg).

Sensitivity to antibiotic was studied on nutrient plate and the respective plate was seeded uniformly by rubbing the swab against the entire agar surface. Each antibacterial impregnated disc was applied onto the surface of the inoculated plate by using sterile forceps. The plates were incubated at 37 °C for 48 hours. The zone of inhibition around each disc was measured and the interpretation was made as per the zone size interpretation chart provided

by the disc manufacturer. The results of sensitivity test was expressed as 3+, 2+, 1+ and R expressing high, moderate, sensitive and resistant, level of susceptibility respectively.

## RESULTS AND DISCUSSION

A cross-sectional study was carried out on mastitis in 108 udder-halves of 54 Black Bengal lactating goats to determine the halves-wise prevalence of clinical and sub-clinical mastitis associated bacterial pathogens and their antibiotic sensitivity patterns in Bangladesh. Each udder of 1025 lactating Black Bengal goats were examined physically in two different districts of Bangladesh, of which only 54 (5.27%) goats were diagnosed as clinical mastitis that constitute the experimental animals for this study. Physical examination of 108 udder-halves of the 54 lactating goats showed 59 (54.63%) udder-halves affected with clinical mastitis (Table 1). Of the 59 affected udder-halves, 49 (90.74%) had unilateral and 5 (9.26%) goats had bilateral clinical mastitis. Significantly ( $p < 0.01$ ) higher prevalence of clinical mastitis was recorded in left udder-halves ( $n = 44$ ; 40.74%) in comparison to right udder-halves ( $n = 11$ ; 10.19%) in Black Bengal goats. These results could not be compared due to lack of similar reports in goats in the available literature. However, these findings support the report of Khan and Muhammad (2005) who reported highest prevalence of clinical mastitis in the left hind-quarter in buffaloes.

Table 1. Udder-half-wise frequency of distribution of bacteria isolated from clinical and sub-clinical mastitis of Black Bengal goats

S/N	Bacterial isolates	Joypurhat district (n = 36; H = 72)		Mymensingh district (n = 18; H = 36)		Total (n = 54; H = 108)		Overall (CH = 59/ SCH = 49) No. + ve (%)
		C/SC (L) No. + ve	C/SC (R) No. + ve	C/SC (L) No. + ve	C/SC (R) No. + ve	C/SC (L) No. + ve	C/SC (R) No. + ve	
①	<i>Staphylococcus</i> spp.	13 / 03	02 / 06	07 / 03	01 / 05	20 / 06	03 / 11	23 / 17 (38.98/34.69)
②	<i>Escherichia coli</i>	10 / 01	01 / 04	05 / 02	00 / 05	15 / 03	01 / 09	16 / 12 (27.12/24.49)
③	<i>Bacillus</i> spp.	00 / 04	04 / 02	02 / 00	00 / 00	02 / 04	04 / 02	06 / 06 (10.17/12.25)
<b>A.</b>	<b>Single infection</b>	23 / 08	07 / 12	14 / 05	01 / 10	37 / 13	08 / 22	45 / 35 (76.27/71.43)
④	<i>Staphylococcus</i> spp. + <i>E. coli</i>	02 / 01	00 / 01	03 / 00	00 / 00	05 / 01	00 / 01	05 / 02 (8.48/4.08)
⑤	<i>Staphylococcus</i> spp. + <i>Bacillus</i> spp.	01 / 02	00 / 01	00 / 00	00 / 00	01 / 02	00 / 01	01 / 03 (1.69/6.12)
⑥	<i>Bacillus</i> spp. + <i>E. coli</i>	00 / 02	03 / 01	01 / 02	00 / 02	01 / 04	03 / 03	04 / 07 (6.78/14.29)
<b>B.</b>	<b>Mixed infection</b>	03 / 05	03 / 03	04 / 02	00 / 02	07 / 07	03 / 05	10 / 12 (16.95/24.49)
	Total positive	26 / 13	10 / 15	18 / 07	01 / 12	44 / 20	11 / 27	55 / 47 (93.22/95.92)
⑦	Total negative	03 / 00	00 / 01	00 / 01	01 / 00	03 / 01	01 / 01	04 / 02 (6.78/4.08)
	Overall	29 / 13	10 / 16	18 / 08	02 / 12	47 / 21	12 / 28	59 / 49

n = Number of goats    H = Number of halves    C = Clinical    SC = Sub-clinical    L = Left halves  
R = Right halves    CQ = Number of clinical halves    SCQ = Number of sub-clinical halves

Bacteriological examination of all the milk samples collected from 108 udder-halves of 54 lactating goats showed 102 (94.44%) udder-halves had intra-mammary infection (IMI). Of the 59 and 49 udder-halves affected clinically and sub-clinically, of which 55 (93.22%) and 47 (95.92%) halves had IMI, respectively (Table 1). It also appears that both the clinical and sub-clinical mastitis affected udder-halves had single (clinical 76.27% and sub-clinical 71.43%) and mixed (clinical 16.95% and sub-clinical 24.49%) bacterial infections (Table 1). No significant difference was recorded on the prevalence of intramammary bacterial infection between the clinically (93.22%) and sub-clinically (95.92%) affected udder-halves. The major bacteria identified were *Staphylococcus* spp. (clinical halves 38.98% and sub-clinical halves 34.69%), *Escherichia coli* (clinical halves 27.12% and sub-

clinical halves 24.49%) and *Bacillus* spp. (clinical halves 10.17% and sub-clinical halves 12.25%) as a single infection, while 16.95% clinical and 24.49% sub-clinical halves had mixed infection with these three organisms (Table 1). These bacterial pathogens have been reported from mastitic goat milk samples elsewhere (Egwu *et al.*, 1994; Contreras *et al.*, 1995; Manser, 1986; White and Hinckley, 1999; Ameh and Tari, 2000; Ndegwa *et al.*, 2000) and in bovine mastitis milk samples in Bangladesh (Siddique *et al.*, 1989; Mahbub-E-Elahi *et al.*, 1996; Kader *et al.*, 2002). Staphylococci are the most important and prevalent mastitis causing pathogen globally including Bangladesh. These organisms are contagious pathogens can be transmitted during unhygienic milking procedures (Menzies and Ramanoon, 2001). Most of the clinical and sub-clinical mastitis cases were caused by *Staphylococcus* spp. (38.98% and 34.69%) which supports the earlier inland report of Rahman and Samad (1984) and elsewhere (Anyam and Adekeye, 1995).

This study recorded highest prevalence of *Staphylococcus* spp in comparison to *E. coli* and *Bacillus* spp. in both the clinical and sub-clinical mastitis quarters which supports the reports of Hunter (1984), Manser (1986), Guha *et al.* (1989), Kalogridou-Vassiliadou (1991), Egwu *et al.* (1994), Ameh *et al.* (1993) and Wakwoya *et al.* (2006). It is recognized that *E. coli* and *Bacillus* spp are the environmental pathogens and their occurrence could be associated with poor hygienic practices in the environment where goats are kept (Burriel, 1997). The high prevalence of coliforms in association with caprine mastitis was consistent with the reports of Moshi *et al.* (1998) and Ameh and Tari (2000) who reported 28.5% and 30% prevalence of coliforms from dairy goats respectively. The high prevalence of coliform infection in both the clinical and sub-clinical quarter infections recorded in this study could be associated with the unclean environment of goats maintained under rural conditions. However, coliform mastitis have been reported to be a rare occurrence in goats in developed world (Hunter, 1984; Manser, 1986; East *et al.*, 1987; Ryan and Greenwood, 1990; Contreras *et al.*, 1995; Smith and Roguinsky, 1997) as the *E. coli* is an environmental pathogens and keeping a clean environment, milking clean, dry teats and avoiding teat end injuries may reduce prevalence of infection (Smith and Sherman, 1994).

The milk samples of four clinically infected quarters yielded no bacterial growth during culture. This could be either due to early infection that had been overcome by the body's defense or the udder may have been infected with organisms that need special media and thus missed in the ordinary course of bacterial isolation (Wakwoya *et al.*, 2006). Furthermore, there are reports indicating that mastitis causing coliforms in 20% of the clinical cases become negative in culturing and coliform infections are usually less than 10 days duration and are rapidly destroyed by inflammatory reactions (Quinn *et al.*, 1999).

The results of *in vitro* antibiotic sensitivity tests of three bacterial isolates to 10 different antibiotics are presented in Table 2. Antibiotic sensitivity results showed moderately to highly sensitivity (2+ to 3+) was recorded in all the tested isolates of *Staphylococcus* spp. to gentamicin, ciprofloxacin, chloramphenicol, enrofloxacin and oxytetracycline, whereas less to moderate sensitivity was obtained with erythromycin (1+ to 2+). Ampicillin, amoxycillin, doxycycline and streptomycin were found resistant to less sensitive (- to 1+). All the tested *E. coli* isolates were found highly sensitive (3+) to gentamicin and ciprofloxacin, moderately to highly

Table 2. Results of *in vitro* antibiotic sensitivity tests of bacterial isolates of milk samples of mastitic goats

S/N	Antibiotics used	Bacterial isolates tested		
		<i>Staphylococcus</i> spp. (n = 10)	<i>Escherichia coli</i> (n = 10)	<i>Bacillus</i> spp. (n = 10)
①	Gentamicin (GN)	2+ to 3+	2+ to 3+	2+ to 3+
②	Ciprofloxacin (CIP)	2+ to 3+	2+ to 3+	2+ to 3+
③	Erythromycin (ERY)	1+ to 2+	2+ to 3+	- to 1+
④	Chloramphenicol (C)	2+ to 3+	1+ to 2+	- to 1+
⑤	Ampicillin (AMP)	- to 1+	- to 1+	-
⑥	Amoxycillin (AMX)	- to 1+	1+ to 2+	-
⑦	Enrofloxacin (ENR)	2+ to 3+	1+ to 2+	2+ to 3+
⑧	Oxytetracycline (OT)	2+ to 3+	2+ to 3+	1+ to 2+
⑨	Doxycycline (DOC)	- to 1+	1+	1+ to 2+
⑩	Streptomycin (SM)	- to 1+	- to 1+	- to 1+

- = Resistance      1+ = Less sensitive      2+ = Moderately sensitive      3+ = Highly sensitive

sensitive (2+ to 3+) to erythromycin and oxytetracycline, moderately sensitive (2+) to chloramphenicol and less sensitive (1+) to doxycycline, whereas resistant to less sensitive (- to 1+) results were obtained with ampicillin and streptomycin. Isolates of *Bacillus* species were found moderately to highly sensitive (2+ to 3+) to gentamicin, ciprofloxacin and enrofloxacin, less to moderate sensitive (1+ to 2+) to oxytetracycline and doxycycline, less sensitive (1+) to chloramphenicol, resistant to less sensitive (- to 1+) to erythromycin and streptomycin, whereas ampicillin and amoxicillin were fully resistant (-) to these isolates. The results of antibiogram studies with 10 different antibiotics against three different isolates of bacteria could not be compared due to lack of similar inland reports on caprine mastitis. However, these results are in consistent with the reports made elsewhere (Egwu *et al.*, 1994; Bhujbal *et al.*, 1999; Wakwoya *et al.*, 2006). Egwu *et al.* (1994) reported the drug resistant to bacterial isolates of coliforms, *Staphylococcus* spp. and *Streptococcus* spp. isolated from mastitic goats in Nigeria. Bhujbal *et al.* (1999) reported the susceptibility of mastitis organisms from goats showed resistant to chloramphenicol (12.2%), gentamicin (21.3%), oxytetracycline (57.6%), ampicillin (78.8%) and amoxicillin (100%). Wakwoya *et al.* (2006) reported *S. aureus* (7.1%), *Bacillus* spp. (37.8%) and *E. coli* (40.4%) resistant to one or more of the antimicrobials tested.

Gentamicin, enrofloxacin and ciprofloxacin and some extent to oxytetracycline are less commonly used for the treatment of mastitis in Bangladesh resulting in higher efficacy of these drugs. Gentamicin and ciprofloxacin proved to be the drug of choice for the treatment of caprine mastitis caused by *Staphylococcus* spp., *E. coli* and *Bacillus* spp. More number of isolates showed resistance to ampicillin, amoxicillin and streptomycin to all these three bacterial pathogens associated with caprine mastitis. Indiscriminate and frequent use of these antibiotics in animals could be the reason for their ineffectiveness against these bacterial pathogens. Since, streptomycin has been extensively used along with penicillin for treating mastitis, it may have led to the development of high resistance in bacteria against this antibiotic.

## CONCLUSIONS

It appears from this study that an overall 5.273% prevalence of clinical mastitis was recorded in goats maintained under rural condition in Bangladesh and its prevalence was found significantly higher in left udder-halves (79.66%) in comparison to the right (20.34%) halves. The high prevalence of intra-mammary infection was also recorded in apparently normal udder-halves (95.92%) of lactating goats those had clinical mastitis in their other quarters. In addition, there was no significant difference on the types of bacterial pathogens between clinically (93.22%) and sub-clinically (95.92%) affected quarters. Antibiotic sensitivity patterns showed gentamicin and ciprofloxacin are the possible effective antibiotics against the major prevalent mastitis pathogens. Examination of udder of lactating goats at regular interval should be done to detect the clinical mastitis and those goats will have clinical mastitis in one quarter should be required similar therapeutic management for both the quarters.

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